Surgical attenuation of spontaneous congenital portosystemic shunts in dogs resolves hepatic encephalopathy but not hypermanganesemia

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Abstract

Aims: Hypermanganesemia is commonly recognized in human patients with hepatic insufficiency and porto-systemic shunting. Since manganese is neurotoxic, increases in brain manganese concentrations have been implicated in the development of hepatic encephalopathy although a direct causative role has yet to be demonstrated. The aim was to evaluate manganese concentrations in dogs with a naturally occurring congenital shunt before and after attenuation as well as longitudinally following the changes in hepatic encephalopathy grade and extent of portosystemic shunting.

Results: Our study demonstrated that attenuation of the shunt resolved encephalopathy, significantly reduced portosystemic shunting yet a hypermanganasemic state persisted.

Conclusion: This study demonstrates that resolution of hepatic encephalopathy can occur without the correction of hypermanganesemia, indicating that increased manganese concentrations alone do not play a causative role in encephalopathy. Our study further demonstrates the value of the canine congenital portosystemic shunt as a naturally occurring spontaneous model of human hepatic encephalopathy.

Running title: Hypermanganesemia after shunt attenuation

Keywords ; canine, shunt, manganese, liver

Introduction

Hepatic encephalopathy (HE) is a common neuropsychiatric syndrome which occurs in patients with liver disease. The pathogenesis of HE is multifactorial with ammonia and inflammation currently considered to be the main causes of neurological disturbances in patients with liver disease. However, other metabolic disturbances, notably in sodium homeostasis, have been linked to the development of HE (Mas 2006, McPhail et al. 2010).

Human patients with liver disease frequently develop hypermanganesemia (Spahr et al. 1996). Dietary manganese (Mn) is efficiently absorbed through the gastrointestinal tract, then extracted by hepatocytes (Schramm and Brandt 1986). Absorbed Mn is then excreted in bile against a high concentration gradient (Klaassen 1974). This process is extremely efficient and only approximately 2% of absorbed Mn ultimately reaches the systemic circulation (Davis et al. 1993). In the situation of reduced hepatic function and portosystemic shunting, high concentrations of Mn are delivered into the systemic circulation (Mizoguchi et al. 2001, Uchino et al. 2007) . As Mn is neurotoxic, hypermanganesemia has been linked to the development of HE (Layrargues et al. 1998).

The pathogenesis of Mn neurotoxicity appears to be multifactorial. Astrocytes accumulate high Mn concentrations, causing release of reactive oxygen species, an increase in peripheral type benzodiazepine receptors and also neurosteroid production (Aschner et al. 1992). Manganese has also been shown to potentiate microglial release of inflammatory mediators in response to LPS and also increase ammonia concentrations in the CNS (Jayakumar et al. 2004, Filipov et al. 2005, Butterworth 2008, Rivera-Mancia et al. 2012).

Despite the established association between liver disease and hypermanganesemia, the precise role of Mn in the development of HE remains unclear. Aside from case reports demonstrating a reduction in globus pallidus hyperintensity, a finding which is pathognomic for Mn deposition, after occlusion of porto-systemic shunts and treatment for hepatic encephalopathy, there is only one study in humans which measured whole blood manganese before and after an improvement in hepatic function (Taguchi et al. 1999, Lazeyras et al. 2002). This study examined Mn concentrations before and after orthotopic liver transplantation (OLT) which failed to demonstrate a significant reduction in Mn concentrations 4 months after transplant. Furthermore, Mn concentrations remained increased in 6 of 12 patients that had increased pre-operative concentrations. In addition, Mn concentrations became increased in a further 3 that had normal concentrations pre-operatively.

Potential explanations for this finding include the persistence of portal-systemic collaterals, recurrence of hepatitis or concurrent disease contributing to high Mn concentrations. However, interpretation of this data is difficult due to the presence of a wide range of comorbidities in the patient cohort. Consequently, there is a clear need to clarify whether the reducing portosystemic shunting reduces Mn concentrations.

One of the most common congenital abnormalities in dogs is a portosystemic shunt (cPSS) which commonly causes spontaneous HE. We have demonstrated that the pathophysiology of HE in dogs with liver disease is very similar to human HE, with ammonia and inflammation playing a key role in the development of neurological abnormalities in dogs with a cPSS (Shawcross et al. 2004, Shawcross et al. 2007, Gow et al. 2012, Tivers et al. 2014). Crucially, we have also demonstrated that dogs with a cPSS frequently have an increase in Mn concentrations (Gow et al. 2010). Consequently, dogs with a cPSS are a highly informative, spontaneous model of human HE in which the pathogenesis of neurological complications associated with liver disorders can be explored without the need for the inducing liver disease in otherwise healthy animals.

In dogs with a cPSS, portosystemic shunting can be surgically attenuated with subsequent resolution of HE in the majority of cases (Hunt and Hughes 1999). This offers an excellent natural experimental system, free from many of the confounding variables which are invariably present in human studies, in which to examine whether the manganese concentrations normalise following surgical attenuation of a portosystemic shunt (Lazeyras et al. 2002). As hepatic blood flow improves after surgery, and hepatic volume increases rapidly, it would be expected that this would allow efficient hepatic first pass extraction of portal blood Mn and systemic concentrations would reduce after shunt ligation (Kummeling et al. 2010). The objective of this study was to test the hypothesis that cPSS attenuation would resolve HE, portosystemic shunting and hypermanganesemia in dogs with a cPSS.

Methods

Dogs with a diagnosis of a cPSS (shunt confirmed by direct visualisation, ultrasonography and/or porto-venography) were enrolled into the study. Whole blood EDTA samples were collected before and after shunt attenuation. A bile acid stimulation test, which is a well established and widely used assay to assess for the presence of portosystemic shunting in dogs, was also performed (Center et al. 1991). At time of sampling, the grade of HE was recorded based on a previously described system (Rothuizen 2009). The whole blood EDTA samples were frozen at -70ºC before being transported on dry ice as a batch to the Scottish Trace Element and Micronutrient Reference Laboratory. Manganese concentrations were determined in whole blood by graphite furnace atomic absorption spectrometry after dilution with Triton X-100 solution. The interassay coefficient of variation was 5.4%. Bile acid measurement was performed on an ILab 650 using a colorimetric, 2 point kinetic fixed time enzymatic reaction by Dialab.

A Wilcoxon matched-pairs test was used to compare HE scores, whole blood Mn and bile acids before and after surgical ligation. Statistical analysis was performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA) and P of <0.05 was considered significant. The study was approved by the University of Edinburgh Veterinary Ethics Committee.

Results

Whole blood Mn concentrations were measured before and after surgery in 26 dogs with a cPSS (2 males neutered, 7 intact males, 14 intact females, 3 females neutered, age range 17 weeks to 5 years 11 months, mean 21 months). All dogs were diagnosed with an extra-hepatic shunting vessel. The median time between sampling was 4 weeks.

Post-prandial bile acid concentrations were significantly lower post-operatively than prior to surgery (median pre-surgery 218µmol/L, media post-surgery 39 µmol/L , p=0.0006). Twelve dogs had signs of hepatic encephalopathy and 14 were asymptomatic prior to surgery. No dog exhibited signs of HE at the time of post attenuation sampling. Hepatic encephalopathy scores were significantly lower at the time of post-operative sampling compared to the pre-operative time point (p=0.0012). There was no significant difference between the pre and post-surgery whole blood Mn measurements (figure 1, median pre-surgery 2969nmol/L, media post-surgery 2296 nmol/L , p=0.12). Manganese concentrations increased in 8 dogs after shunt attenuation.

Discussion

The central finding of this study was that clinical signs of HE resolved and portosystemic shunting significantly decreased following shunt attenuation yet, whole blood manganese concentrations did not decrease significantly. Indeed, in eight individuals Mn concentrations increased. This finding is consistent with the OLT study where Mn concentrations remained increased after liver transplantation (Lazeyras et al. 2002).

The reason(s) why Mn concentrations remain high in dogs post cPSS attenuation is unclear. Dogs with a cPSS have reduced hepatic volume and post-operatively, despite improved portal delivery of Mn to the liver, a lack of functional hepatocytes may initially prevent efficient extraction of Mn (Stieger et al. 2007). Hepatic volume has been shown to increase rapidly post-operatively with the greatest increase between days 0 to 8 post attenuation, thus it would be expected that efficiency of portal Mn extraction would likewise increase (Kummeling et al. 2010). As Mn distribution is reasonably uniform through most body tissues, cPSS cases would have tissue Mn to equilibrate with whole blood concentrations, also slowing post-operative resolution of hypermanganesaemia (Dobson et al. 2004). It has also been demonstrated that systemic Mn is less efficiently extracted by the liver than portally delivered Mn (Teeguarden et al. 2007). This may be due to difference in oxidation state; the majority of portally delivered Manganese is Mn^{2+} however during systemic circulation this can be oxidised by ceruloplasmin to Mn^{3+} and bound to transferrin (Teeguarden et al. 2007) .

Longer-term monitoring of whole blood Mn in cases of cPSS which have undergone surgical attenuation would be useful to assess if concentrations gradually decrease. Persistent hypermanganesemia after clinically successful surgery may have significant clinical consequences with continued CNS Mn deposition causing subtle long term neurological abnormalities that current canine HE scoring systems cannot detect. Long term follow up of neurological status and ultimately examination of CNS Mn concentration and histopathological examination would be insightful.

In summary, this study demonstrated that dogs with a congenital porto-systemic shunt are an instructive natural model in which Mn homeostasis can be probed, free from many of the confounding factors present in humans undergoing OLT and without the need to induce disease in otherwise healthy animals. Despite clinical resolution of HE and a significant reduction in portosystemic shunting, hypermanganesemia remained present in these dogs.

Conflict of interest: The authors declare that they have no conflicts of interest

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

Aschner, M., M. Gannon and H. K. Kimelberg (1992). "Manganese uptake and efflux in cultured rat astrocytes." J Neurochem 58(2): 730-735.
Butterworth, R. F. (2008). "Pathophysiology of hepatic encephalopathy: The concept of synergism." Hepatol Res 38 Suppl 1: S116-121.
Center, S. A., T. ManWarren, M. R. Slater and E. Wilentz (1991). "Evaluation of twelve-hour preprandial and two-hour postprandial serum bile acids concentrations for diagnosis of hepatobiliary disease in dogs." J Am Vet Med Assoc 199(2): 217-226.
Davis, C. D., L. Zech and J. L. Greger (1993). "Manganese metabolism in rats: an improved methodology for assessing gut endogenous losses." Proc Soc Exp Biol Med 202(1): 103-108.
Dobson, A. W., K. M. Erikson and M. Aschner (2004). "Manganese neurotoxicity." Ann N Y Acad Sci 1012: 115-128.
Filipov, N. M., R. F. Seegal and D. A. Lawrence (2005). "Manganese potentiates in vitro production of proinflammatory cytokines and nitric oxide by microglia through a nuclear factor kappa B-dependent mechanism." Toxicol Sci 84(1): 139-148.
Gow, A. G., A. I. Marques, D. A. Yool, K. Crawford, S. M. Warman, P. D. Eckersall, R. Jalan and R. J. Mellanby (2012). "Dogs with congenital porto-systemic shunting (cPSS) and hepatic encephalopathy have higher serum concentrations of C-reactive protein than asymptomatic dogs with cPSS." Metab Brain Dis 27(2): 227-229.
Gow, A. G., A. I. Marques, D. A. Yool, A. Duncan and R. J. Mellanby (2010). "Whole blood manganese concentrations in dogs with congenital portosystemic shunts." J Vet Intern Med 24(1): 90-96.
Hunt, G. B. and J. Hughes (1999). "Outcomes after extrahepatic portosystemic shunt ligation in 49 dogs." Aust Vet J 77(5): 303-307.
Jayakumar, A. R., K. V. Rama Rao, P. Kalaiselvi and M. D. Norenberg (2004). "Combined effects of ammonia and manganese on astrocytes in culture." Neurochem Res 29(11): 2051-2056.

Klaassen, C. D. (1974). "Biliary excretion of manganese in rats, rabbits, and dogs." Toxicol Appl Pharmacol 29(3): 458-468.
Kummeling, A., D. J. Vrakking, J. Rothuizen, K. M. Gerritsen and F. J. van Sluijs (2010). "Hepatic volume measurements in dogs with extrahepatic congenital portosystemic shunts before and after surgical attenuation." J Vet Intern Med 24(1): 114-119.
Layrargues, G. P., C. Rose, L. Spahr, J. Zayed, L. Normandin and R. F. Butterworth (1998). "Role of manganese in the pathogenesis of portal-systemic encephalopathy." Metab Brain Dis 13(4): 311-317.
Lazeyras, F., L. Spahr, R. DuPasquier, J. Delavelle, P. Burkhard, A. Hadengue, D. Hochstrasser, G. Mentha, E. Giostra, F. Terrier and F. Vingerhoets (2002). "Persistence of mild parkinsonism 4 months after liver transplantation in patients with preoperative minimal hepatic encephalopathy: a study on neuroradiological and blood manganese changes." Transpl Int 15(4): 188-195.
Mas, A. (2006). "Hepatic encephalopathy: from pathophysiology to treatment." Digestion 73 Suppl 1: 86-93.
McPhail, M. J., J. S. Bajaj, H. C. Thomas and S. D. Taylor-Robinson (2010). "Pathogenesis and diagnosis of hepatic encephalopathy." Expert Rev Gastroenterol Hepatol 4(3): 365-378.
Mizoguchi, N., Y. Nishimura, H. Ono and N. Sakura (2001). "Manganese elevations in blood of children with congenital portosystemic shunts." Eur J Pediatr 160(4): 247-250.
Rivera-Mancia, S., C. Rios and S. Montes (2012). "Manganese and ammonia interactions in the brain of cirrhotic rats: effects on brain ammonia metabolism." Neurochem Res 37(5): 1074-1084.
Rothuizen, J. (2009). "Important clinical syndromes associated with liver disease." Vet Clin North Am Small Anim Pract 39(3): 419-437.
Schramm, V. L. and M. Brandt (1986). "The manganese(II) economy of rat hepatocytes." Fed Proc 45(12): 2817-2820.
Shawcross, D. L., N. A. Davies, R. Williams and R. Jalan (2004). "Systemic inflammatory response exacerbates the neuropsychological effects of induced hyperammonemia in cirrhosis." J Hepatol 40(2): 247-254.
Shawcross, D. L., G. Wright, S. W. Olde Damink and R. Jalan (2007). "Role of ammonia and inflammation in minimal hepatic encephalopathy." Metab Brain Dis 22(1): 125-138.
Spahr, L., R. F. Butterworth, S. Fontaine, L. Bui, G. Therrien, P. C. Milette, L. H. Lebrun, J. Zayed, A. Leblanc and G. Pomier-Layrargues (1996). "Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms." Hepatology 24(5): 1116-1120.
Stieger, S. M., A. Zwingenberger, R. E. Pollard, A. E. Kyles and E. R. Wisner (2007). "Hepatic volume estimation using quantitative computed tomography in dogs with portosystemic shunts." Vet Radiol Ultrasound 48(5): 409-413.
Taguchi, Y., S. Takashima, S. Hirade and H. Inoue (1999). "[Disappearance of globus pallidus hyperintensity in a patient with portal-systemic encephalopathy after occlusion of the shunt vessel]." Rinsho Shinkeigaku 39(5): 565-569.
Teeguarden, J. G., D. C. Dorman, A. Nong, T. R. Covington, H. J. Clewell, 3rd and M. E. Andersen (2007). "Pharmacokinetic modeling of manganese. II. Hepatic processing after ingestion and inhalation." J Toxicol Environ Health A 70(18): 1505-1514.
Tivers, M. S., I. Handel, A. G. Gow, V. J. Lipscomb, R. Jalan and R. J. Mellanby (2014). "Hyperammonemia and systemic inflammatory response syndrome predicts presence of hepatic encephalopathy in dogs with congenital portosystemic shunts." PLoS One 9(1): e82303.
Uchino, A., T. Noguchi, K. Nomiyama, Y. Takase, T. Nakazono, J. Nojiri and S. Kudo (2007). "Manganese accumulation in the brain: MR imaging." Neuroradiology 49(9): 715-720.

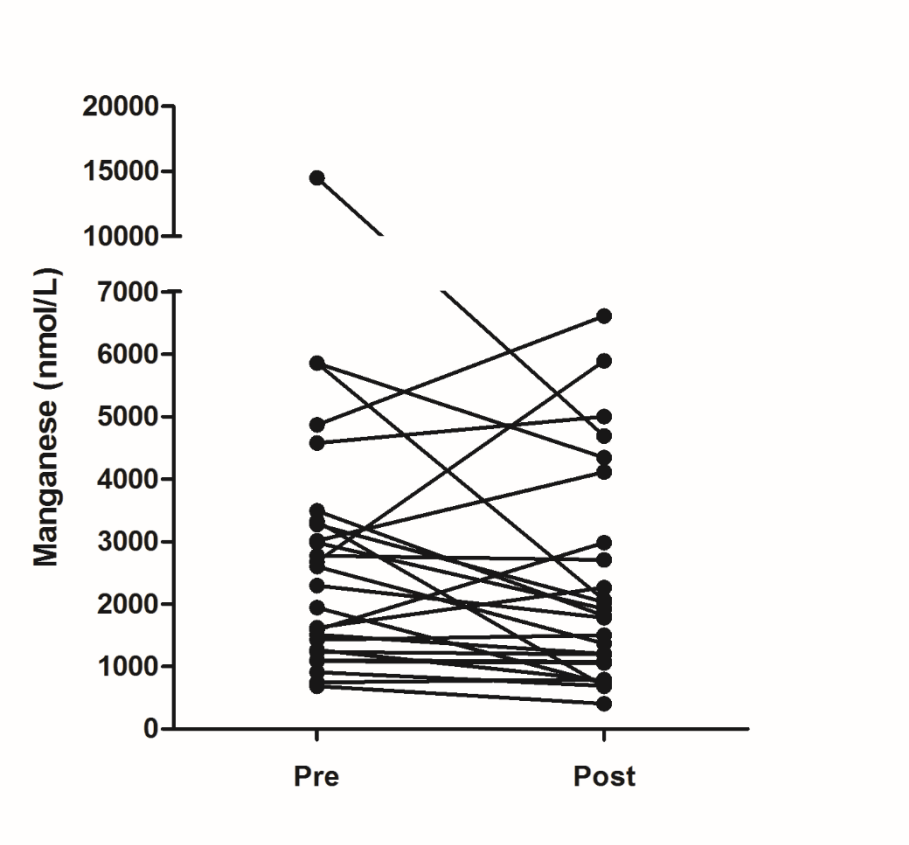

Figure 1: Whole blood manganese concentrations of 26 dogs with a congenital portosystemic shunt before (Pre), and after (Post) surgical shunt ligation. There was no significant difference in concentrations before and after ligation (p=0.12).